**PROJECT NUMBER :** 6906
**PROJECT TITLE :** Biological Effects of Smoke
**PROJECT LEADER :** G. J. Patskan
**PERIOD COVERED :** December, 1990

## I. JB6 MOUSE EPIDERMAL CELL TRANSFORMATION ASSAY (G. Nixon)

A. **Objective:** To obtain a colony formation response to CSC treatment in soft agar, and to select a new serum stock lot.

B. **Results:** Two soft agar assays testing the effects of 2R1 CSC and 12-0-tetradecanoylphorbol-13-acetate (TPA) in combination were completed. In both experiments, the combination of treatments resulted in lower colony counts than when TPA was used by itself. This may be a toxicity effect, as colony counts were lower in both promotion-sensitive and transformed cells when TPA and CSC were combined. Acetone was compared to dimethyl sulfoxide (DMSO) as a possible solvent for CSC in the soft agar assay. The two solvents were comparable in control plates (TPA), but neither was effective in supporting colony formation of the promotion-sensitive JB6 cells by CSC in soft agar. Two new lots of Hyclone serum were put into soft agar tests. The new lot which supported cell growth and colony formation in soft agar (using TPA treatment) most comparable to the current stock lot has been obtained in large quantities for use as the stock lot for the next year.

C. **Plans:** The logistics of testing whole smoke in the soft agar assay will be studied.

D. **References:**

Burruss, T.J. Notebook No. 8896, p. 34.

Vaughan, B.G. Notebook No. 8458, p. 109

Nixon, G. M. Notebook No. 8711, p. 165.

## II. SALMONELLA/MICROSOME (S/M) ASSAY

A. **Objective:** To test the biological activity of experimental CSCs and other pertinent materials.

B. **Results:** Several CSC samples were tested in support of the Crossed Solubles Base Web (CSBW) study. CSCs from experimental cigarettes prepared by individuals external to the Biochemical Research Division were also tested.

Additionally, data was analyzed to evaluate a "screening" protocol for the S/M Assay.

C. **Plans:** Test samples for biological activity as they become available. Document a "screening" protocol for the S/M Assay.

D. **References:**

Jones, R. Notebook No. 8769, p. 100.

Stagg, D. Notebook No. 9038, p. 49.

## III. PLANT TISSUE CULTURE (M. Shulleeta)

A. **Objective:** To develop procedures for the establishment, maintenance and transformation of plant cell cultures.

B. **Results:** Studies were conducted to optimize both the salt concentration and osmoticum for purification, evacuolation and culture of Burley 21 protoplasts. In preliminary experiments a complex medium (8P) with 0.33M sucrose as osmoticum was identified as an appropriate medium for use in tobacco cell transformation.

Microcalli were successfully generated from Burley 21 protoplasts even though these protoplasts had undergone spontaneous evacuolation due to the suboptimal medium conditions used in a previous purification procedure. These microcalli have been plated onto standard callus proliferation medium following successive dilution into osmoticum-free medium.

Callus material generated from the routine cell culture of Burley 21 stem tissue has been transferred to regenerative medium to verify the capability of this cell line to form intact plantlets.

Burley 21 callus material cultured on a nicotine production medium (RM2) was tested via a Dragendorff squash-blot procedure to verify the presence of alkaloids in the tissue. The results indicated that alkaloids were produced by the RM2-grown callus in contrast to callus grown on standard proliferative medium which do not produce detectable levels of alkaloids.

C. **Plans:** Repeat Burley 21 protoplast purification and culture using 8P medium with 0.33M sucrose as osmoticum.

Assess the regenerability of Burley 21 calli cultured from evacuolated and non-evacuolated protoplasts.

Initiate suspension cultures of Burley 21 cells and determine the growth kinetics.

Electroporate Burley 21 protoplasts with PZO plasmid (coding for hygromycin resistance and GUS production) and assay for transient expression of GUS.

D. **References:**

Shulleeta, M. Notebook No. 8961.